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Acute and Subchronic Toxicity Studies of Methanol Leaves Extract of *Ficus Platyphylla* on Albino Rats

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ABSTRACT

F. platyphylla is a tree that is native throughout the tropics with a few extending into the semi-warm temperate zone. In Nigeria, it is predominant in the Northern regions of the country. Acute oral toxicity (LD_{50}) was determined using five (5) rats and monitored for fourteen while twenty five (25) rats were used for the subchronic toxicity studies. They were grouped into five groups and treated daily with the extract orally for 28 days. The estimated LD50 was found to be greater than 3000mg/kg body weight as no mortality was recorded during the 14 days observation period. The biochemical indices of the kidney and liver on different doses of the extract were not significantly different ($P>0.05$) except for creatinine, ALT and AST which showed significant decrease ($P<0.05$) when compared to control. However, haematological parameters such as WBC and PLT were also significantly increased ($P<0.05$) compared to the control. The findings reveal *F. platyphylla* may be potentially safe for consumption.

Keywords: *Ficus Platyphylla,* Acute Toxicity, Subchronic Toxicity, Albino Rat

1. INTRODUCTION

The use of traditional medicine in developed as well as developing countries as basis for the treatment of many ailments has been in existence for thousands of years and there is no doubt that their importance has been widely acknowledged (Chukwuma *et al.*, 2015). Schmelzer and Gurib-Fakin (2008) emphasized that medicinal plants significantly contribute to rural livelihoods of the people and social equilibrium in Africa. Chukwuma *et al.* (2015) reported that about 80% of the population in Nigeria use traditional medicine. Traditional medicine enjoys a wider acceptability among the people of developing countries partly due the fact it blends readily into the socio-cultural life of the people in whose culture it is deeply rooted and also due to the inaccessibility of orthodox medicine (Sofowora *et al.*, 2013). In Africa, the resolution on "Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region", adopted by the fiftieth meeting of the

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WHO Regional Committee for Africa in August 2000, states that the African Member States are aware that about 80% of the region's population depends on traditional medicine for its health care needs (WHO, 2019). Some reasons advanced for this include affordability accessibility and beliefs (Trim *et al.*, 2005).

Toxicity testing of new compounds is essential for drug development process (Ekor, 2014). It has been shown that the toxicity of a given plant depends on various factors, including the strength of secondary metabolites, the quantity consumed, the time of exposure, different parts of the plant (root, oil, leaves, stem bark and seeds), individual body chemistry, climate and soil, and genetic differences within the species (Chaachouay *et al.*, 2021). There is therefore the need to assess the toxicity levels of these plants.

2. MATERIAL AND METHODS

Plant collection and identification

Fresh leave of *F. platyphylla* were collected in February, 2020 from Zuru Local Government, Kebbi State, Nigeria. This plant was authenticated by a taxonomist from the Department of Plant Biology and Biotechnology, Kebbi State University of Science and Technology, Aliero where a voucher specimen (122A) was deposited in the herbarium.

Extraction of plant materials

F. platyphylla leaf collected was washed with tap water and air dried at room temperature under shade. It was pounded to coarse powder using a mortar and pestle. One thousand two hundred grams (850g) was macerated with 2500ml of methanol for 72hrs. It was filtered and the filtrate evaporated in an electric oven at 45ºC. The dried extract was stored in clean airtight containers and kept in a refrigerator at 8^0C until it was required to be used.

Experimental Animals

Thirty (20) female albino rats used for the study were procured from Animal House, Usmanu Danfodiyo University, Sokoto in February, 2020. They were transported in well-ventilated cages to Animal House, Faculty of Life Science, Kebbi State University of Science and Technology, Aliero. The rats were housed in clean cages and were allowed to acclimatize for fourteen (14) days before the commencement of the experiment.

Acute Oral Toxicity Study (LD_{50})

The acute oral toxicity study was conducted according to the method of Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001). A total of five (5) animals were randomly selected and used for the experiment. A single oral limit test dose of 3000 mg/kg body weight was administered. The animals were observed for signs of drowsiness, hair loss, loss of appetite, salivation and tremors. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity.

Sub-chronic Toxicity Studies

The wistar albino rats were randomly grouped into five (5) groups of five (5) rats each. Group 1 served as the control group receiving distilled water. Group 2 – 5 were orally treated daily with methanol extract of *Ficus platyphylla* at 150 mg/kg, 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight respectively for 28 days.

Biochemical Analysis

The rats were fasted overnight on the 28^{th} day and on the 29^{th} day, weights were taken after which they were sacrificed, blood samples were collected via cardiac puncture and centrifuged at 3000rpm for 10mins to obtain serum for further analysis. Biochemical parameters measured were serum alanine amino transferase (ALT) and aspartate amino transferase (AST) (Reitman and Frankel, 1957), total proteins (Gornall *et al.*, 1949), Bilirubin (Total and direct) (Malloy and Evelyn, 1937), alkaline phosphatase (Wright *et al.*, 1972), urea (Young, 1997), and creatinine (Bartels and Bohmer, 1971), levels using biochemical assay kits while serum electrolytes sodium and potassium would be measured using flame photometry (Cheesbrough, 1991).

Haematological analysis

Parked Cell Volume (PCV), Haemoglobin concentration, Red Blood Cells count (RBC), White Blood Cells count (WBC) (neutrophils, lymphocytes, eosinophils, monocytes, basophiles), Haematocrit (HCT), Platelets, Mean Cell Haemoglobin Concentration (MCHC)

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and Mean Cell Haemoglobin (MCH) were analysed using an automated haematological analyser (Sysmex XS800i, Sysmex corporation, USA).

Statistical analysis

All data were presented as Mean ± Standard Error of Mean (SEM). Statistical software SPSS 20.0 was utilized. The results were statistically analysed using a one-way analysis of variance (ANOVA) test. Statistical differences of $P<0.05$ were considered to be significant.

3. RESULTS AND DISCUSSION

Extraction of *F. platyphylla* 850g leaf in 2500mls of methanol yielded 12.67% and the extract was soluble in water, black in colour and with a sticky texture. Single oral administration of 3000mg/kg of methanol extract of *F. platyphylla* on albino rats did not produce any visible signs or symptoms of toxicity in the treated animals there was also no mortality recorded. The LD_{50} of MLEFP was found to be greater than 3000mg/kg body weight as no mortality was recorded in any of the experimental rats.

The liver biomarkers of toxicity assayed revealed a significant ($P<0.05$) decrease in AST in all the treated groups when compared to the normal control (Table 1). ALT decreased significantly ($P<0.05$) at 600 and 1200mg/kg body weight treated groups when compared with the normal control group. The results of renal function biomarkers (Creatinine, Urea, Uric Acid, Na, K, Cl^- and HCO_3^-) are presented in Table 2. There was significant elevation ($P<0.05$) elevation in creatinine (300, 600 and 1200mg/kg body weight) and urea (1200mg/kg body weight) levels. K^+ and Cl^- levels decreased significantly ($P<0.05$) at higher doses while Na^+ decreased significantly ($P<0.05$) at higher doses too. Methanol leaf extract of *F. platyphylla* did not cause significant changes in Red Blood Count, Haemoglobin, Mean Cell Haemoglobin Concentration, Mean Cell Haemoglobin, Mean Corpuscular Volume and Packed Cell Volume (Table 3). There was significant decrease ($P<0.05$) in platelet count in all the treated groups when compared to the normal control group while there was significant increase ($P<0.05$) in white blood count in all treated groups (except for that which received the lowest dose) when compared to the normal control group.

Table 1: Effect of crude methanol leaf extract of *F. platyphylla* on biomarkers of liver function on albino Rats

Parameter	Methanol Leaf Extract of *F. platyphylla*				
	Control	(150mg/kg)	(300mg/kg)	(600 mg/kg)	(1200 mg/kg)
TP (G/l)	82.80±1.39[a]	80.40±1.03[a]	81.20±2.60[a]	79.40±2.02[a]	80.00±1.61[a]
ALB (G/l)	52.80±1.66[a]	49.20±3.15[a]	46.80±2.25[a]	48.60±0.87[a]	47.60±2.79[a]
TB (mg/dL)	0.64±0.05[a]	0.70±0.05[a]	0.66±0.21[a]	0.48±0.04[a]	0.48±0.02[a]
DB (mg/dL)	0.40±0.06[b]	0.20±0.03[a]	0.23±0.02[a]	0.25±0.03[a]	0.29±0.03[a]
AST (U/L)	149.20±6.04[c]	111.60±2.34[ab]	123.40±3.78[b]	105.00±12.26[ab]	56.80±3.57[a]
ALT (U/L)	40.60±1.44[a]	36.60±4.76[a]	30.60±4.76[ab]	26.20±0.37[b]	21.00±3.05[c]
LP (U/L)	25.60±2.23[a]	25.20±1.50[a]	27.40±1.60[a]	32.60±3.01[b]	27.00±2.63[a]

Values are presented as mean ± standard error of mean, n = 5, mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test).

AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin and DB- Direct Bilirubin

Table 2 Effect of crude methanol leaf extract of *F. platyphylla* on biomarkers of kidney function on albino Rats

Parameter	Methanol Leaf Extract of *F. platyphylla*				
	Control	(150mg/kg)	(300mg/kg)	(600 mg/kg)	(1200 mg/kg)
Creatinine (mg/dl)	1.28 ± 0.01[a]	1.43 ± 0.09[a]	1.50 ± 0.07[a]	1.59 ± 0.07[b]	1.63 ± 0.01[b]
Urea (mmol/l)	7.21 ± 0.11[a]	7.43 ± 0.45[a]	7.50 ± 0.37[a]	7.50 ± 0.04[a]	7.73 ± 0.02[b]
Uric acid (mg/dl)	2.89 ± 0.06[a]	2.97 ± 0.60[a]	3.13 ±0.06[a]	3.16 ±0.02[a]	3.71 ± 0.02[a]

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HCO_3^-(mmol/l)	20.64 ± 0.51 [a]	21.63 ± 0.71 [a]	22.61 ± 0.73 [a]	21.34 ± 0.32 [a]	22.96 ± 0.40 [a]
Na^+ (mmol/l)	139.73 ± 0.57 [b]	136.18 ±1.53 [b]	133.97 ± 1.38 [b]	129.62 ± 0.88 [a]	125.71 ± 1.91 [a]
K^+ (mmol/l)	5.57 ± 0.04 [a]	5.69 ± 0.05 [a]	5.78 ± 0.04 [a]	6.73 ± 0.08 [b]	6.29 ± 0.09 [b]
Cl^- (mmol/l)	91.03 ± 0.44 [a]	92.05 ± 1.07 [a]	93.47 ± 1.89 [a]	96.6 ± 1.38 [a]	97.81 ± 1.48 [a]

Values are presented as mean ± standard error of mean, n = 5, mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test).
Potassium (K+), Sodium (Na+), Bicarbonate (HCO_3)

Table 3: Effect of crude methanol leaf extract of *F. platyphylla* on haematological Indices on albino rats

Doses (mg/kg)	**RBC (10^{12}/L)**	**MCHC (g/dL)**	**MCH (pg)**	**MCV (fL)**	**HGB (g/dL)**	**PCV (%)**	**PLT ($x10^9$/L)**	**WBC ($x10^9$/L)**
Control	7.06±0.81	30.50±1.40	19.33±2.19	61.13±0.95	12.80±0.29	42.60±0.80	716.33±91.41	6.99±0.85
150	7.11±0.59	30.30±0.59	18.37±0.57	60.73±3.05	13.03±0.95	43.07±3.45	529.00±60.37*	10.44±0.40
300	7.65±0.06	30.37±0.43	18.50±0.00	60.93±0.84	14.13±0.12	42.53±2.60	526.33±75.97*	11.03±1.36*
600	7.10±0.64	29.80±0.46	18.23±0.38	61.17±0.46	12.90±0.92	43.37±3.71	520.00±55.77*	12.61±1.28*
1200	7.19±0.14	30.70±0.52	18.93±0.34	61.73±1.89	13.60±0.26	44.37±0.67	626.67±40.29*	14.90±2.07*

Values are presented as mean ± standard error of mean, n = 5, mean values having common superscript letters in a column are not significantly different (P<.05) (one-way ANOVA followed by Duncan's multiple range test).
RBC- Red Blood Count, MCHC-Mean Cell Haemoglobin Concentration, MCH- Mean Cell Haemoglobin, MCV- Mean Corpuscular Volume, HGB- Haemoglobin, PCV- Packed Cell Volume, PLT- Platelets, WBC- White Blood Count.

The index of acute toxicity is the LD_{50} measured in mg/kg bw. It is usually the first step in the evaluation of the toxic characteristics of a substance (Ogbuehi *et al.*, 2015). The globally harmonized system (GHS) of classification and labelling of hazardous chemicals set out five categories for single dose toxicity test. A substance whose LD_{50} is in the range of 2000-5000 mg/kg bw, is placed in category 5 and may be harmful if swallowed (GHS, 2015). The LD_{50} of MLEFP therefore suggests that it is essentially non-fatal and non-toxic if swallowed and is therefore safe in oral formulation.

Changes in ALT and AST activities may indicate alteration of cellular permeability or cellular injury and necrosis. Elevated activities of AST is also observed in myocardial infarction Low (Crook, 2006). The increase in ALT level in serum of albino rats administered with MLEFP could be an indication that it could alter the cellular permeability of the liver thereby by leading to necrosis.

Albumin and total proteins are globular proteins found in the serum and they are synthesized by the liver. A decrease in albumin and total protein is a sign of reduced synthetic ability of the liver or might be due to impaired hepatocellular function (Yakubu *et al.*, 2003). In the present study, the absence of alteration in albumin and total protein is suggestive that the synthetic ability of the liver was not affected. Bilirubin results from the breakdown of haemoglobin and it is excreted out of the body by the liver (Oboh, 2005). Higher than normal levels of bilirubin may indicate different types of liver problems as well as an increased rate of destruction of RBC. In the present study, no significant changes in total and direct bilirubin in the serum of rats treated with MLEFP, indicating that MLEFP did not interfere with the metabolism of bilirubin in the liver (Yakubu *et al.*, 2003).

Urea and uric acid are the major nitrogen containing metabolic end product of protein catabolism. Creatinine is a waste product of muscle energy metabolism (Panda, 1999). Since the results of serum levels of urea, creatinine and uric acid of this study were not altered in all the extract treated groups, it could be deduced that MLEFP might not have exerted any hazardous effect on the kidneys and hence it can possibly be considered as potentially safe for use as a medicinal plant (Iglesias and Diez, 2008). Increased sodium in the blood may result due to kidney disease, too little water intake, and loss of water due to diarrhoea or vomiting. Decrease in sodium levels can result from relative increase in the amount of body water relative to sodium. This could lead to respiratory arrests, neurogenic pulmonary oedema, and hypoxic exacerbation of cerebral oedema leading to fatal herniation (Sterns and Hix, 2009). Potassium ion is a cation of the intracellular fluid which plays a vital role in muscle contraction (Parikh and Webb, 2012). The most common cause of high potassium is kidney disease. Other causes may result from dehydration, uncontrolled diabetes and injuries that cause severe bleeding (Parham *et al.*, 2006). The effect of MLEFP at higher doses investigated on the renal

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function biomarkers may suggest that the normal functionality of the nephrons at the tubular and glomerular levels can be altered by high concentrations of MLEFP. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered (Enemor and Okaka, 2013). Chloride ion is involved in maintaining proper water distribution, osmotic pressure and normal acid-base balance in the extracellular fluid compartment. The lack of alteration in serum chloride and bicarbonates implies that the acid-base balance was not affected upon treatment with MLEFP (Shirley *et al.*, 2003).

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals (Odeyemi *et al.*, 2009). Blood-relating functions of a plant extract or its products can be explained through the indices of this system. Since changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies, analysis of this becomes applicable and relevant in toxicity studies (Ukwuani *et al.*, 2012). The total red blood cell count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), haemoglobin, and PCV are the most useful indicators in the diagnosis of anaemia in humans and animals (Akpamu *et al.*, 2011). Adebayo *et al.*, (2017) reported that reduction in RBC, HB and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anaemia. On the contrary an increase in the count of RBC, HB and PCV is suggestive of polycythaemia and positive erythropoiesis (Okpuzor *et al.*, 2009). In the present study the evaluation of total RBC, MCHC, MCH, MCV, haemoglobin and PCV did not show significant changes following repeated administration of crude methanol leaf extract of *F. platyphylla* at all the tested doses as compared to the controls. These results are in agreement with other findings in which the values of the various haematological parameters of extract treated rats were found to be comparable with those of the control (Okpuzor *et al.*, 2009; Yakubu and Afolayan, 2009). These results indicate that there is no lysis of blood cells, bleeding, anaemia and inhibition in blood cells synthesis or bone marrow suppression by any of the active constituents in the crude methanol leaf extract of *F. platyphylla*.

White blood cell count increases in response to foreign substance as a defence mechanism of the body (Adebayo *et al.*, 2017). Increase in WBC count may also be due to enhancement of WBC production and reduction in its removal from the circulation in an attempt to defend the system (Okpuzor *et al.*, 2009). On the other hand, a low white blood cell count could be as a result of a viral infections that temporarily disrupt the work of bone marrow, cancer or any other diseases that damage bone marrow, severe infections that use up white blood cells faster than they are produced or medications, such as antibiotics, that destroy white blood cells (Ukwuani *et al.*, 2012). The rise in WBC in this study, therefore suggests that methanol leaf extract of *F. platyphylla* contributes in potentiating the defence system.

According to Roberts and Murray (2003), platelets which are fragments of cells, participate in blood clotting and are considered as an acute phase reactant to infection or inflammation. Reduction in platelet number has also been associated with infections, deficiency of folate or vitamin B12, drugs and some herbal remedy (Ukwuani *et al.*, 2012). Increase in platelet count is could be as a result of anaemia due to iron deficiency or infection. Thrombocytopenia otherwise known as low platelet count may result from vitamin deficiencies, increased platelet destruction caused by drugs such as heparin, sequestration caused by enlarged spleen or pregnancy (Roberts and Murray, 2003). Decrease in platelet count was observed in the rats treated with crude methanol leaf extract of *F. platyphylla* at all the tested doses suggests that the chemical constituents of the crude methanol leaf extract of *F. platyphylla* may cause thrombocytopenia.

4. CONCLUSION

From the acute and sub chronic toxicity studies carried out, the *F. platyphylla* can be used in for varied medicinal purposes as it did not exhibit adverse effects on the albino rats.

Authors' contributions

This work was carried out in collaboration among all authors. Author Angela Nnenna Ukwuani-Kwaja conceptualized designed and supervised the study. Author Lesley Sahber Kindzeka performed the experiment collected all data, and wrote the first draft of the manuscript. Author Ibrahim Sani did the literature search performed the statistical analysis and wrote the final manuscript. All authors read and approved the final manuscript.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

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Ethical approval

The Animal ethical guidelines are followed in the study for experimentation. The ethical guidelines for plants & plant materials are followed in the study for experimentation.

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Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Adebayo MA, Enitan SS, Owonikoko WM, Igogo E, Ajeigbe KO. Haematinic properties of methanolic stem bark and fruit extracts of Ficus Sur in rats pre-exposed to phenylhydrazine-induced haemolytic anaemia. African Journal of Biomedical Research. 2017 May 5;20(1):85-92.
2. Akpamu U, Nwaopara AO, Izunya AM, Oaikhena GA, Okhiai O, Idonije BO, Osifo UC. A comparative study on the acute and chronic effect of oral administration of Yaji (a complex Nigerian meat sauce) on some hematological parameters. British Journal of Pharmacology and Toxicology. 2011 Aug 5;2(3):108-12.
3. Bartels H, Böhmer M. Eine Mikromethode zur Kreatininbestimmung [Micro-determination of creatinine]. Clin Chim Acta. 1971 Mar;32(1):81-5. German. doi: 10.1016/0009-8981(71)90467-0. PMID: 5096431.
4. Chaachouay N, Benkhnigue O, Douira A, Zidane L. Poisonous medicinal plants used in the popular pharmacopoeia of the Rif, northern Morocco. Toxicon. 2021 Jan 15;189:24-32. doi: 10.1016/j.toxicon.2020.10.028. Epub 2020 Nov 10. PMID: 33181163.
5. Cheesbrough M. Medical laboratory manual for tropical countries. M. Cheesbrough, 14 Bevills Close, Doddington, Cambridgeshire, PE15 OTT.; 1981.
6. Chukwuma EC, Soladoye MO, Feyisola RT. Traditional medicine and the future of medicinal Plants in Nigeria. Journal of Medicinal Plants Studies. 2015;3(4):23-9.
7. Crook M. Clinical chemistry & metabolic medicine. 7th Edition. Hodder Arnold, Londo. 2006 p.426.
8. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014 Jan 10;4:177. doi: 10.3389/fphar.2013.00177. PMID: 24454289; PMCID: PMC3887317.
9. Enemor VH, Okaka AN. Sub-acute effects of ethanol extract of Sarcocephalus latifolius root on some physiologically important electrolytes in serum of normal Wistar albino rats. Pak J Biol Sci. 2013 Dec 1;16(23):1811-4. doi: 10.3923/pjbs.2013.1811.1814. PMID: 24506054.
10. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949 Feb;177(2):751-66. PMID: 18110453.
11. Iglesias P, Díez JJ. Thyroid dysfunction and kidney disease. Eur J Endocrinol. 2009 Apr;160(4):503-15. doi: 10.1530/EJE-08-0837. Epub 2008 Dec 18. PMID: 19095779.
12. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. Journal of Biological Chemistry. 1937 Jul 1;119(2):481-90.
13. Oboh G. Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (Telfairia occidentalis) leaves against garlic-induced oxidative stress. J Med Food. 2005 Winter;8(4):560-3. doi: 10.1089/jmf.2005.8.560. PMID: 16379574.
14. OECD O. Guidelines for testing of chemicals, acute oral toxicity-fixed dose procedure. Organ. Econ. Coop. Dev. 2001.
15. Odeyemi OO, Yakubu MT, Masika PJ, Afolayan AJ. Toxicological evaluation of the essential oil from Mentha longifolia L. subsp. capensis leaves in rats. Journal of medicinal food. 2009 Jun 1;12(3):669-74.
16. Ogbuehi IH, Ebong OO, Obianime AW. Oral acute toxicity (LD50) study of different solvent extracts of Abrus precatorius Linn leaves in wistar rats. European Journal of Experimental Biology. 2015;5(1):18-25.
17. Okpuzor, J., Ogbunugafor, H. A., and Kareem, G. K. Hepatic and hematologic effects of fractions of *Globimetula braunii* in normal albino rats. *Experimental and Clinical Sciences*. 2009 *8*:182-9.
18. Parham WA, Mehdirad AA, Biermann KM, Fredman CS. Hyperkalemia revisited. Tex Heart Inst J. 2006;33(1):40-7. PMID: 16572868; PMCID: PMC1413606.
19. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957 Jul;28(1):56-63. doi: 10.1093/ajcp/28.1.56. PMID: 13458125.
20. Schmelzer GH, Gurib-Fakim A, editors. Medicinal plants. Prota; 2008.

ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

21. Shirley DG, Unwin RJ, Haycock GB, van't Hoff WG, Adrogué HJ, Madias NE. The patient with. Oxford Textbook of Clinical Nephrology Volume 2. 2005;2:921.
22. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines. 2013 Aug 14;10(5):210-29.
23. Sterns RH, Hix JK. Overcorrection of hyponatremia is a medical emergency. Kidney Int. 2009 Sep;76(6):587-9. doi: 10.1038/ki.2009.251. PMID: 19721422.
24. Trim SA, Trim CM, Williams HF, Vaiyapuri S. The Failures of Ethnobotany and Phytomedicine in Delivering Novel Treatments for Snakebite Envenomation. Toxins (Basel). 2020 Dec 6;12(12):774. doi: 10.3390/toxins12120774. PMID: 33291263; PMCID: PMC7762085.
25. Ukwuani A, Ihebunna O, Samuel RM, Peni IJ. Acute oral toxicity and antiulcer activity of Piliostigma thonningii leaf fraction in albino rats. Bull Env Pharmacol Life Sci. 2012;2:41-5.
26. United Nations. Economic Commission for Europe. Secretariat. Globally harmonized system of classification and labelling of chemicals (GHS). Copyright Law of the United St; 2015.
27. World Health Organization. WHO global report on traditional and complementary medicine 2019. World Health Organization; 2019 May 16.
28. Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine: alkaline phosphatase. Enzymologia. 1972 Apr 28;42(4):317-27. PMID: 4337755.
29. Yakubu MT, Afolayan AJ. Effect of aqueous extract of Bulbine natalensis Baker stem on haematological and serum lipid profile of male Wistar rats. Indian J Exp Biol. 2009 Apr;47(4):283-8. PMID: 19382725.
30. Young DS. Effects of drugs on clinical laboratory tests. Ann Clin Biochem. 1997 Nov;34 (Pt 6):579-81. doi: 10.1177/0 00456329703400601. PMID: 9366995.

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